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Deworming of intestinal helminths reduces HIV-1 subtype C viremia in chronically co-infected individuals

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SUMMARY

Objective: To define the impact of helminthic infestations and their treatment on viral load and T cell subsets in chronic HIV-1-infected patients.
Methods: Two hundred twenty chronic HIV-1-infected Ethiopian patients with (N = 87) and without (N = 133) helminthic infestations were included. To determine the impact of deworming on viral load and T cell subsets, a subset of these patients with (n = 23) and without (n = 20) helminthic infestations were followed longitudinally. Helminth egg loads, plasma HIV RNA levels, and peripheral blood $CD4^+$ and $CD8^+$ T cells were determined at baseline and at 12 weeks after antihelminthic treatment.
Results: At baseline, plasma viral load was significantly higher in individuals with (n = 87) than without (n = 133) a helminthic infestation (5.01 $\log_{10}$ vs. 3.41 $\log_{10}$, $p < 0.001$). Twelve weeks after antihelminthic treatment, plasma HIV RNA levels were reduced in the successfully treated group ($p < 0.001$). Twelve weeks after antihelminthic treatment, helminth infestations and their treatment had no significant effect on $CD4^+$ T cell counts. However, helminth-infested individuals had a higher level of $CD8^+$ T cells at baseline ($p < 0.001$), which was significantly reduced ($p < 0.01$) at 12 weeks after antihelminthic treatment.
Conclusions: Helminths were found to be associated with an increased HIV RNA level. Successful treatment of intestinal helminths reduced plasma HIV RNA levels in chronic HIV-1 subtype C infection. Considering the high endemicity of helminths in tropical settings, the management of chronically HIV-infected individuals must include deworming.

1. Introduction

Since the beginning of the HIV/AIDS epidemic in 1981, nearly 30 million people have died of AIDS-related causes,[1] and today, three decades later, the virus has spread throughout the world. There are approximately 34 million people living with HIV/AIDS. However, in the last decade, global efforts to address this epidemic have shown some success and there are signs that the epidemic may be changing course. Among others, the number of people with HIV receiving treatment in resource-poor countries has increased more than 26-fold since 2003, reaching eight million in 2011 (54% antiretroviral therapy (ART) coverage in the region). This has averted a total of 2.5 million deaths. Nevertheless, Sub-Saharan Africa bears a disproportionate burden of the HIV epidemic. It is reported by the World Health Organization (WHO) that 69.1% of people living with HIV reside in this region. Co-infection with other pathogens like helminths, malaria, and tuberculosis, which are still rampant in the region,[2] may accelerate the transmission and pathogenesis of HIV by enhancing viral replication.

It is well documented that helminths have a profound effect on the host immune system and that these effects may spill over to impact on immune responses to other pathogens[3,4] by inducing dominant type 2 response profiles, which inhibit the type 1 response profile needed to combat and control viral antigens such as HIV.[5] Accordingly, it has been hypothesized that the chronic immune activation and the presence of a dominant Th2 cytokine environment may increase the risk of acquiring HIV infection[6] and increase the plasma HIV RNA level.[7,8] However, the impact of intestinal helminths and their treatment on the HIV viral load and T cell count among asymptomatic HIV-infected individuals remains controversial. For example, a significant association between helminth intensity and plasma HIV viral load, with a significant decrease in HIV-1 viral load following deworming, has been reported.[8] Moreover, eradication of *Ascaris lumbricoides* from asymptomatic HIV-1-infected subjects resulted in a significant elevation in $CD4^+$ T cell counts and a trend towards a plasma HIV-1

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viral load decrease after 12 weeks of follow-up.[9] In contrast, the results of several other studies do not support the above evidence.[10–12] Data on the role of helminth infestations and their treatment in chronic HIV infection are lacking. The purpose of the current study was therefore to examine the impact of helminthic infestations and their treatment on the HIV-1 plasma RNA level and T cell count during chronic HIV infection.

2. Methods

2.1. Study design and patients

A prospective observational study was conducted to investigate the effect of helminth infestations and their treatment on plasma HIV-1 RNA levels and T cell counts at the University of Gondar teaching hospital, in the north-west of Ethiopia, from April to October 2008. HIV-infected persons over 18 years of age, with/without advanced HIV disease, seeking treatment and willing to participate, were evaluated using a standardized form at enrolment. Ethical clearance was obtained from the Ethics Committee of the University of Gondar and informed consent was obtained from all study subjects. The WHO criteria for AIDS-defining conditions were used.[13] Patients were excluded for the following reasons or conditions: pregnancy, treatment with single-dose nevirapine for prevention of mother-to-child transmission of HIV or any other ART, known diabetes, hypertension, epilepsy, liver, cardiac, and renal diseases, genital ulcers, or active tuberculosis at enrolment. In total, 220 consecutive HIV-1-infected treatment-naïve individuals with advanced HIV disease (WHO clinical stages 3 and 4)[13] were enrolled and screened for intestinal parasites. Patients who started ART at enrolment (n = 130) or during the follow-up period (n = 43) were excluded (Figure 1). Patients at WHO clinical stage 4, irrespective of their $CD4^+$ T cell count, those at WHO clinical stage 3 with a $CD4^+$ T cell count $\leq$350 cells/mm^3, and patients with a $CD4^+$ T cell count $\leq$200 cells/mm^3 at any WHO clinical stage, were eligible for ART.

2.2. Stool examination

Fresh stool samples collected at enrolment and at 12 weeks after antihelminthic treatment were examined by direct microscopy technique using normal saline and iodine (to identify trophozoites and cysts of protozoan parasites) and by formalin–ether sedimentation and Kato–Katz concentration methods (to detect helminth eggs and larvae). Coarse quantification of helminth eggs (expressed as eggs per gram of feces) was carried out in accordance with the Kato–Katz technique and a scoring system (light, moderate, and heavy infection) was adopted from the WHO recommendations.[14] Antihelminthic treatment was given to all study participants with an intestinal parasite infestation: 400 mg albendazole once for those with *A. lumbricoides*, *Trichuris trichiura*, and hookworm; 200 mg albendazole twice daily for 3 consecutive days for those with *Strongyloides stercoralis*; and 40 mg/kg praziquantel for those with *Schistosoma mansoni*). Albendazole 400 mg once was also given to all individuals without evidence of a helminth infestation, as there might be false-negative results and to control its effect (if any).

2.3. T cell count

Venous blood (5 ml) was collected in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) at enrolment and at 12 weeks after antihelminthic treatment for T cell subset counts and plasma HIV RNA viral load determination. T cell counts were done using a flow cytometer (FACSCount system; Becton Dickinson, San Jose, CA, USA) following the manufacturer's protocol.

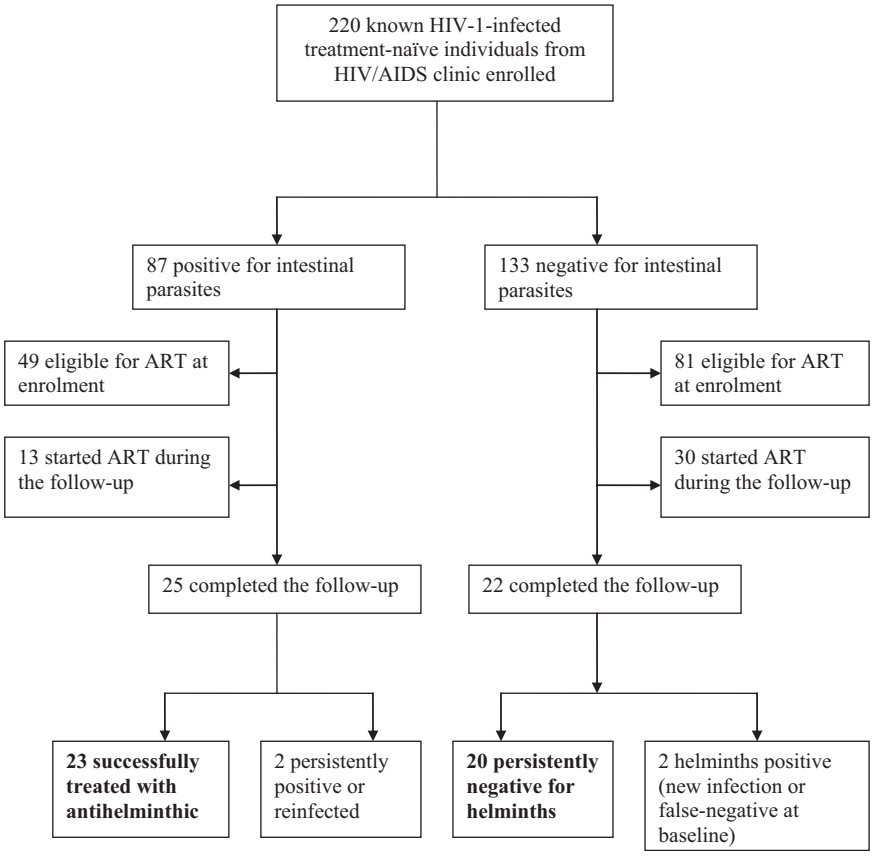

Figure 1. Flow chart of HIV-1-infected individuals with and without helminth infections included in the study.

2.4. Determination of plasma HIV-1 RNA level

The plasma HIV-1 RNA level was determined by quantitative real-time PCR (Abbott m2000rt instrument, Abbott Molecular, Des Plaines, IL, USA). The lower detection limit of the assay was 40 copies/ml (1.6 $\log_{10}$ RNA copies/ml). HIV-1 genotyping was undertaken following an in-house protocol (Mulu et al., 2012, submitted for publication). Briefly, a set of nested primers was used for amplification of the protease and reverse transcriptase region of the HIV-1 genome and sequencing was carried out using an ABI Prism 310 (Applied Biosystems, Foster City, CA, USA).

2.5. Statistical analysis

The data were analyzed using SPSS software version 17. Baseline characteristics of the individuals with helminth infestations (N = 87) and those without (N = 133) were compared using the Student's t-test. Egg counts and plasma HIV RNA levels were $\log_{10}$-transformed for normalizing dispersed values. Changes in plasma HIV RNA levels and T cell counts at baseline and at 12 weeks after antihelminthic treatment were compared by paired t-test in successfully dewormed patients (n = 23) and in patients who were persistently negative for helminths (n = 20). A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics of the subjects

Two hundred twenty chronically HIV-1-infected individuals were screened for intestinal parasites (92 male and 128 female). The rate of helminth infestation among males and females was not significantly different (45% vs. 35%). There was no significant difference in age among females and males, and in the prevalence of intestinal parasites by age, sex, and other socio-demographic variables. Eighty-seven individuals were found to be infected with one or more intestinal parasites, making the overall prevalence of intestinal parasites 39.5% (87/220). *A. lumbricoides* (24; 10.9%), *T. trichiura* (14; 6.4%), hookworm (12; 5.5%), and *S. mansoni* (10; 4.5%) were the most prevalent species detected. Single infestations and multiple infestations (double and triple parasitic infestations) were detected in 72 (32.7%) and 15 (6.8%) subjects, respectively.

3.2. Baseline differences in $CD4^+$ and $CD8^+$ T cell counts and plasma HIV RNA levels

At enrolment, there was no significant difference in $CD4^+$ T cell counts between helminth-infested and non-infested patients (214 vs. 212 cells/mm^3, respectively). However, the mean $CD8^+$ T cell count was significantly higher in those with a helminth infestation than in those without a helminth infestation (970 $\pm$ 113 cells/mm^3 vs. 893 $\pm$ 97 cells/mm^3, p = 0.035). The mean plasma HIV viral load was consistently and significantly higher in individuals with lower $CD4^+$ T cell levels. The mean plasma HIV RNA level at baseline (n = 220) was 4.30 $\pm$ 1.09 $\log_{10}$ RNA copies/ml. There were no differences in plasma HIV RNA levels (p = 0.7) and $CD4^+$ T cell counts (p = 0.3) between females and males. However, plasma HIV RNA levels were significantly higher in individuals co-infected with intestinal parasites (n = 87) than in those not co-infected (n = 133) (4.83 $\pm$ 0.9 vs. 3.95 $\pm$ 1.1 $\log_{10}$ HIV RNA copies/ml, $p < 0.001$).

No significant association was found between the presence of an individual intestinal parasite and plasma HIV viral load. Individuals infested with multiple intestinal parasite species (n = 15) had slightly higher mean plasma HIV RNA levels than those infested with single parasites (n = 72) (4.83 $\pm$ 0.78 vs. 4.62 $\pm$ 0.75 $\log_{10}$ RNA copies/ml, $p < 0.01$). No significant association ($p > 0.05$) was found between helminth egg loads and plasma HIV RNA levels. The mean baseline plasma HIV RNA level was not significantly different between individuals with high or moderate intensity helminth infestations (n = 12) and in individuals with low intensity helminth infestations (n = 48) (4.21 $\pm$ 0.83 vs. 4.14 $\pm$ 0.48 $\log_{10}$ copies/ml, $p > 0.05$). HIV-1 subtype C was identified in all cases (data not shown).

In the helminth treated and responder group, similar to the entire study group, there was no significant difference in $CD4^+$ T cell count (Figure 2A) between helminth-infested and non-infested patients (358 $\pm$ 92 vs. 323 $\pm$ 78 cells/mm^3, respectively). However, at baseline the $CD8^+$ T cell count, as well as HIV viral load, was significantly higher in the helminth-infested group compared to the non-infested group (1413 $\pm$ 161 vs. 896 $\pm$ 124 cells/mm^3; 5.01 vs. 3.41 $\log_{10}$ copies/ml) (Figure 2B and 2C). There was no significant association between helminth egg load and plasma HIV RNA level (data not shown). There were no statistically significant changes in $CD4^+$ T cell counts between baseline and 12 weeks after anti-helminthic treatment and the presence of specific parasites (Table 1).

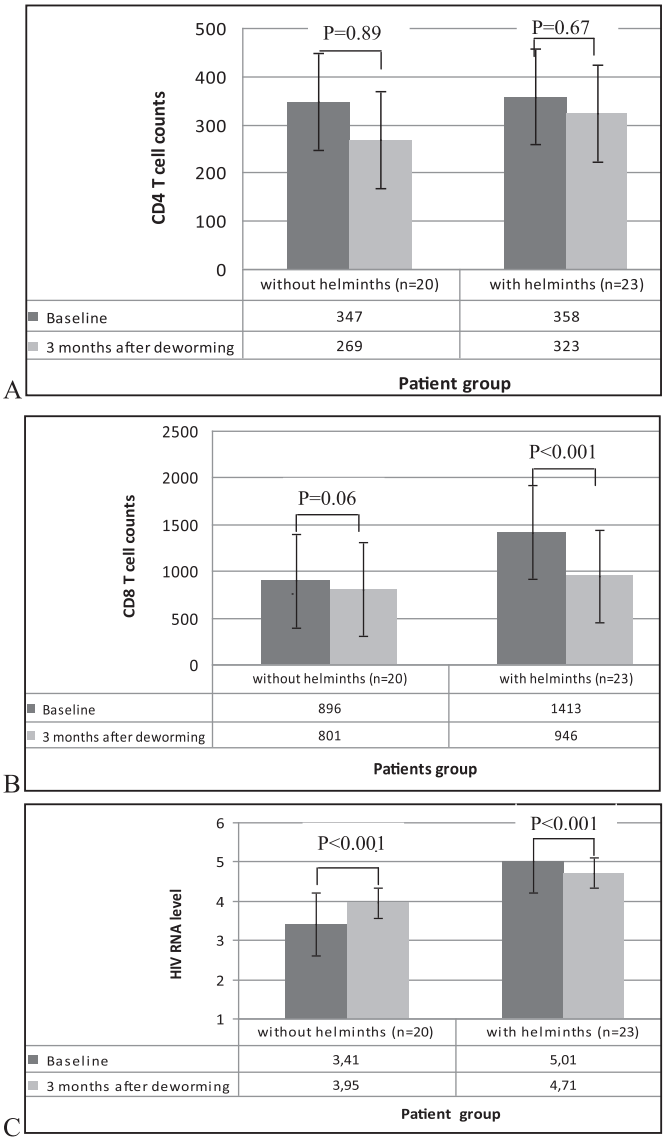

Figure 2. Changes in plasma HIV RNA levels and $CD4^+$ and $CD8^+$ T cell counts in patients with and without helminths before and after antihelminthic treatment (HIV RNA level in $\log_{10}$ copies/ml and T cells in number/mm^3).

Table 1
Helminth species-specific changes in HIV RNA levels (log_{10} HIV RNA in copies/ml) and $CD4^+$ and $CD8^+$ T cell counts (cells/mm^3) before and after deworming

Helminth	Baseline	After 12 weeks	*p*-Value
Helminths successfully treated			
Ascaris lumbricoides (*n* = 10)			
Log_{10} HIV RNA	4.94 ± 0.85	4.74 ± 0.85	0.001[a]
$CD4^+$ T cell count	386	365	0.070
$CD8^+$ T cell count	1469	1073	0.001[a]
Hookworm (*n* = 6)			
Log_{10} HIV RNA	4.94 ± 0.69	4.75 ± 0.67	0.038[a]
$CD4^+$ T cell count	363	314	0.06
$CD8^+$ T cell count	1108	978	0.029[a]
Trichuris trichiura (*n* = 4)			
Log_{10} HIV RNA	5.22 ± 0.48	4.91 ± 0.49	0.086
$CD4^+$ T cell count	394	366	0.69
$CD8^+$ T cell count	1442	1361	0.051
Strongyloides stercoralis (*n* = 1)			
Log_{10} HIV RNA	5.6	4.55	NA
$CD4^+$ T cell count	347	245	NA
$CD8^+$ T cell count	1066	931	NA
Schistosoma mansoni (*n* = 1)			
Log_{10} HIV RNA	4.80	4.71	NA
$CD4^+$ T cell count	294	237	NA
$CD8^+$ T cell count	1344	1139	NA
Hookworm and *S. stercoralis* (*n* = 1)			
Log_{10} HIV RNA	4.96	4.82	NA
$CD4^+$ T cell count	363	219	NA
$CD8^+$ T cell count	1384	1196	NA
Persistently helminth-positive			
S. mansoni (*n* = 1)			
Log_{10} HIV RNA	3.08	6.19	NA
$CD4^+$ T cell count	350	321	NA
$CD8^+$ T cell count	2000	1679	NA
S. stercoralis (*n* = 1)			
Log_{10} HIV RNA	4.82	5.31	NA
$CD4^+$ T cell count	291	236	NA
$CD8^+$ T cell count	1744	1355	NA

NA, not applicable.
[a] $p < 0.05$.

3.3. Impact of helminth treatment on the plasma HIV RNA level and $CD4^+$ and $CD8^+$ T cell counts

The effect of helminth treatment on HIV viral load and $CD4^+$ and $CD8^+$ T cell counts was assessed in 47 patients who were not on antiretroviral drugs (25 with a helminth infestation and 22 without). Additional stool and blood samples were collected 12 weeks after antihelminthic treatment. Among the 25 individuals with a helminth infestation, 23 were successfully treated (92%) and only two (8%) were found to be positive for the same helminth at the end of 12 weeks.

Twelve weeks after antihelminthic treatment, a significant reduction ($p < 0.001$) in $CD8^+$ T cells was observed (Figure 2B) in the treated and responder group. Interestingly, 12 weeks after antihelminthic treatment, there was a mean decrease in plasma HIV RNA level (-0.3 log_{10} ± 0.83) in the successfully treated and responder group (5.01 vs. 4.71 log_{10} copies/ml; $p < 0.001$) (Figure 2C). In contrast, there was a statistically significant mean increase in plasma HIV RNA level (+0.54 log_{10} ± 0.23) in the persistently helminth-negative group (Figure 2C). The change in plasma HIV RNA among individuals with high (≥5.0 log_{10} copies/ml; *n* = 17) baseline plasma HIV RNA was -0.25 log_{10} copies/ml after antihelminthic treatment in the helminth-infested group (5.24 vs. 4.99 log_{10} copies/ml; $p = 0.3$) and +0.02 log_{10} copies/ml at the same time point in the helminth non-infested group (5.18 vs. 5.16 log_{10} copies/ml; $p = 0.8$). The primary outcome was not different after adjusting for age, sex, and $CD4^+$ T cell count (data not shown). Irrespective of the type of intestinal helminth, there was a decline in plasma HIV RNA level at 12 weeks after antihelminthic treatment. A significant reduction in plasma HIV RNA level was observed in individuals infested with Ascaris and hookworm (Table 1).

4. Discussion

The finding of a 0.88 log_{10} difference in baseline HIV viral load between individuals with a helminth co-infection and those without is in line with previous studies from East African countries.[8–10,15] Although a higher HIV viral load has been reported to be associated with faster disease progression[16–18] in the context of helminthic infestations, the concept remains controversial due to a lack of well-controlled longitudinal studies[19] and because of other potential factors that could contribute to the increase in plasma HIV RNA level.[4,20,21] A study from Zambia showed a higher median pre-treatment plasma HIV RNA level (0.33 log_{10} copies/ml) in the helminth uninfected group compared to the helminth infested group.[12] Studies from Malawi and Uganda have reported a lack of association between helminth infestation and faster progression of HIV disease in co-infected individuals.[11,22] Nevertheless, the higher HIV viral load among chronically infected HIV patients with a helminth co-infection compared to helminth uninfected patients could be due to immune activation in the helminth infestation, resulting in a higher level of $CD8^+$ T cells.[21,23] It has been documented that helminth infestations alter T cell subsets and that the treatment of helminth infestations shows a tendency to reduce the immune activation, indicating that the treatment of helminth infestations could be used to down-regulate the observed immune activation.[23,24] Similarly, an increased $CD8^+$ lymphocyte count and elevated levels of activated $CD8^+$ T cells expressing HLA-DR were observed among Ethiopian immigrants with helminth infestations in Israel,[21] suggesting that immune activation is a major factor in increasing HIV-1 RNA levels, accounting for the increased susceptibility and progression of HIV infection in Africa and the developing countries.[3]

An impact on $CD4^+$ T cell numbers was not detectable in individuals with or without a helminth co-infection.[8,25] The overall observed low level of $CD4^+$ T cell counts in the current study is in agreement with previous reports.[8,25] It has to be remembered that irrespective of the HIV infection state, $CD4^+$ T cell counts are lower among Ethiopians than among people from the USA or from Europe.[23,26] The prevalence of co-infection with intestinal parasites among chronically infected HIV-1 subtype C patients was as high as 39.5%, which corresponds well with previous studies from Sub-Saharan Africa.[12,15,16,27]

An increase in plasma HIV RNA level among helminth uninfected individuals within 12 weeks was observed, reflecting the natural history of HIV disease progression in ART-naïve individuals.[19] The significant decline (-0.3 log_{10}) in plasma HIV RNA levels after deworming of helminths in the infested and responder group in this study is in line with a systematic review of trials by Walson et al.,[16] who suggested that the treatment of helminth species may reduce the plasma HIV RNA load. Taking the natural course of HIV infection into account, the decline in plasma HIV viral load as a consequence of the deworming treatment in the present study is impressive. It is consistent with the data from a systematic review.[16] Similarly, the treatment of other co-infections, e.g. malaria and sexually transmitted diseases, has long been known to significantly reduce the HIV viral load.[28–30] In contrast to the findings in chronic HIV, studies of asymptomatic HIV-infected individuals did not indicate an association between treatment of intestinal helminth infestations and a decrease in plasma HIV RNA levels.[10–12] The difference in plasma HIV RNA levels cannot be attributed to albendazole, since individuals without evidence of a helminth infestation received this broad-spectrum antihelminthic drug at baseline and it did not cause a decrease in plasma HIV RNA.

A correlation between helminth infestation intensity and the plasma HIV RNA level has previously been reported.[8,9] However, we could not find such a correlation in our study population, probably because of a lower burden of helminths or variable duration of helminth infestation, which limits the excretion of eggs.[31] The lack of association of plasma HIV viral load and the presence of specific helminths in this study are in agreement with a previous study,[8,12,15] but differ from a study conducted in Kenya that reported a significant association between HIV RNA level and ascariasis.[9] Why HIV was impacted by ascariasis but not by others remains unclear. All studies discussed in this paper were conducted on subjects with asymptomatic HIV infections. Thus, the observed differences in the findings could also be related to both the variation in HIV subtypes and the course of HIV infection.

In conclusion, despite its obvious limitations such as the small sample size, the data presented here are of significant public health importance in areas where both HIV and helminths coexist epidemiologically. Chronically HIV-1 subtype C infected individuals with helminth co-infections had a significantly higher level of HIV viremia compared to helminth uninfected individuals (4.83 ± 0.9 vs. 3.95 ± 1.1 $\log_{10}$ HIV RNA copies/ml, $p < 0.001$) and deworming significantly reduced the level of plasma HIV RNA (5.01 vs. 4.71 $\log_{10}$ copies/ml; $p < 0.001$). Considering the prevalence of helminths in tropical settings and the possible implication of HIV–helminth co-infection, diagnosis and treatment of helminth infestations is an important approach to contribute to delaying HIV-1 disease progression, as has previously been suggested.

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